Biotherapeutics for Mitigation of Health Disorders from *Terminalia arjuna*

Field of Invention

The present invention relates to a series of metabolites, which were isolated from *Terminalia arjuna* and characterized for therapeutically relevant molecules to be used in treatment of various health related disorders like cardiovascular disorders and as antibacterial agents. The extracts from the plants were subjected to both, targeted and non-targeted screening procedures. The present invention also relates to a series of extracts exhibiting significant antioxidant potential, as indicated by the DPPH free radical scavenging and reducing potential, thereby extrapolating to its cardio-protective function. The broad-spectrum activity of *Terminalia arjuna* extracts against the microorganisms tested potentates its application as an anti-microbial agent.

Background

Indian folk medicine comprises numerous herbal prescriptions for therapeutic purposes which may be as varied as healing wounds, treating inflammation due to infection, skin lesions, leprosy, diarrhoea, scabies, veneral diseases, snake bite and ulcers etc.

Terminalia arjuna is a deciduous tree found throughout India growing to a height of around 60-90 feet. *Terminalia arjuna* belongs to the family *Combretaceae* and is called "Arjuna" in vernacular. *Terminalia arjuna* has been used for over 1500 years in India as a cardio tonic and has been indicated for derangement of all three humoursin, vata, pitta and kapha in Ayurveda. The bark of Terminalia arjuna has been widely used in Indian system of medicine for a variety of purposes.

Cardiovascular disease (CVD) is a leading cause of mortality and is responsible for the deaths of around 17 million people every year, accounting for approximately one-third of deaths. Of the estimated deaths attributed to CVD worldwide, 80% is in developing countries. [1]

Lifelong production of free radicals through normal function of mitochondria and other cellular constituents is a major intrinsic source of oxidative stress in the body. The DNA in each cell of a normal rat produces <100000 oxidative lesions per day. DNA repair enzymes are constantly removing these, but with age, the balance shifts toward a higher rate of damage than repair, leading to accumulation of DNA lesions, cell dysfunction, and, in rats, death within 2–3 years. Human cells, in contrast, produce about one-tenth as many oxidative lesions as rat cells and excrete fewer damaged DNA products. Thus, they have a relatively better repair process and antioxidant defense mechanism, which is consistent with the longer life span of humans.[2]
In addition to endogenous oxidative stress, exposure to free radicals and oxidants in the environment, such as ultraviolet sunlight, ozone, cigarette smoke, smog, and other pollutants, also contribute substantially to the rate of

change in the body's oxidant: antioxidant balance. A shift in the oxidant: antioxidant balance due to increased production of free radicals may contribute to the decline of cardiovascular, neuronal, muscular, visual, and immune functions, over time. In addition, a high level of oxidative stress and free radicals has been implicated in an ever-widening array of age-related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract.[2]

Epidemiological studies have demonstrated an association between increased intake of antioxidant molecules and reduced morbidity and mortality from cardiovascular disorders.

Atherosclerosis, a chronic inflammatory disease of the arterial wall, is a major cause of morbidity and mortality from cardiovascular disease (CVD) in much of the world's population. There have been several reports indicating oxidation of Low Density Lipoprotein (LDL) as one of the major mechanisms responsible for the pathogenesis of atherogenesis. The hypothesis that oxidative stress plays a role in atherosclerosis rests on the inference based on experimental work, on a large scale, carried out in animal models of heart disease and by extension, antioxidants by their ability to quench free radicals and reactive oxygen species, may play a beneficial role in modulating oxidative damage and thereby decreasing the risk of atherosclerotic lesion formation and progression.[3]

Nitric oxide (NO) is produced from L-arginine in the vascular endothelium by the endothelial iso-form of nitric-oxide synthase (NOS). Endothelial production of NO is crucial in the control of vascular tone, arterial pressure, smooth muscle cell proliferation and platelet adhesion to the endothelial surface. Impaired endothelium-derived NO bioactivity is a common feature of many vascular diseases that is thought to contribute to their clinical manifestations, as evidenced in a study conducted to investigate the effect of ascorbic acid on NO synthesis. The study also revealed that ascorbic acid was shown to enhance impaired endothelium-dependent vasodilatation in patients with atherosclerosis by a mechanism that is thought to involve protection of NO from inactivation by free oxygen radicals. Ascorbate pretreatment on endothelial cells led to a 3-fold increase of the cellular production of NO measured as the formation of its co-product citrulline and as the accumulation of its effector molecule cGMP. It was thus shown that intracellular ascorbic acid enhances NO synthesis in endothelial cells and that this may explain, in part, the beneficial vascular effects of ascorbic acid.[4, 5]

Cigarette smoking has been recognized as a primary risk factor for cardiovascular diseases, the major cause of morbidity and mortality in industrialized nations. Cigarette smoke introduces a high burden of radicals into the organism and also stimulates the generation of further radicals and reactive oxygen species from activated leukocytes, many of which are chemotactically attracted and sequestered in the lungs. The increased burden of reactive species in the plasma of smokers results in the consumption of the antioxidant vitamin C, and subsequently the accumulation of various lipid

oxidation products in their bloodstream. It has been shown that oxidation of synthetic, cellular, and lipoprotein phosphatidylcholine all create a similar spectrum of phospholipid products that activate inflammatory cells through the platelet-activating factor (PAF) receptor.

Dietary supplement with the antioxidant Vitamin C has shown to prevent the accumulation of PAF like lipids, and also prevented cigarette smoke-induced leukocyte adhesion to the vascular wall and formation of leukocyte-platelet aggregates.[6]

Low plasma vitamin C concentration has been related to increased progression of carotid atherosclerosis and increased risk of acute myocardial infraction. Few studies have also shown an association between plasma vitamin C levels and the risk of stroke. It was seen that low serum or plasma ascorbic acid has been associated with increased incidence of stroke and mortality from stroke.[7]

References:

1. Integrated Management of Cardiovascular risk, Report of a WHO meeting Geneva, 9-12 July 2002.
2. Meydani M. Effect of functional food ingredients: Vitamin E modulation of cardiovascular diseases and immune status in the elderly, Am J Clin Nutr 2000; 71 (suplI): 1665S-8S.
3. Meydani M. Vitamin E and Atherosclerosis: Beyond prevention of LDL oxidation, J. Nutr. 131: 366S-368S, 2001.
4. Regine Heller, Felix Munscher-Paulig, Rolf Grabner, and Uwe Till, L-Ascorbic acid potentates nitric oxide synthesis in endothelial cells, J. Biol. Chem. Vol.274, No.12, Issue of March 19, pp. 8254-8260, 1999.
5. Annong Huang, Joseph A. Vita, Richard C. Venema, and John F. Keaney, Jr. Ascorbic acid enhances endothelial nitric-oxide synthase activity by increasing intracellular tetrahydrobiopterin, J. Biol. Chem. Vol.275, No. 23, Issue of June 9, pp.17399-17406, 2000.
6. Thomas M. Mcintyre et al. Vitamin C blocks inflammatory platelet-activating factor mimetics created by cigarette smoking, J. Clin. Invest. Vo.99, No.10, May 1997, 2358-2364.
7. J. T. Salonen et al., Plasma vitamin C modifies the association between hypertension and risk of stroke, Stroke. 2002; 33:1568-1573.

Prior Art

US patent 6,162,438 describes an edible herbal compositions, mixture of at least three, preferably at least six, herbs selected from the group consisting of *Terminalia arjuna, Cynara scolymus, Zingibar officinale, Allium sativum, Crataegus oxycantha, Curcuma longa, Boerhaavia diffusa* and *Trigonella foenumgraecum*, for use as agents for the control of hypertension, hypercholesterolemia and hyperlipidemia in mammals.

Sharma VN et al. evaluated the antioxidant and hypocholesterolaemic effects of *Terminalia arjuna* tree bark (a popular cardiotonic substance in Indian pharmacopoeia) and compared it with a known antioxidant, vitamin E by a randomised controlled trial. One hundred and five successive patients with coronary heart disease (CHD) were recruited and using a Latin-square design, divided into 3 groups of 35 each. At baseline, total cholesterol, triglycerides, HDL and LDL cholesterol and lipid peroxide estimated as thiobarbituric acid reactive substances (TBARS) were determined. Group I received placebo capsules; Group II vitamin E capsules 400 units/day; and Group III received finely pulverized *T. arjuna* tree bark-powder (500 mg) in capsules daily. Lipids and lipid peroxide levels were determined at 30 days follow-up. It was found that response rate in various groups varied from 86% to 91%. No significant changes in total, HDL, LDL cholesterol and triglycerides levels were seen in Groups I and II (paired t-test $p > 0.05$). In Group III there was a significant decrease in total cholesterol (-9.7 +/- 12.7%), and LDL cholesterol (-15.8 +/- 25.6%) (paired t-test $p < 0.01$). Lipid peroxide levels decreased significantly in both the treatment groups ($p < 0.01$). This decrease was more in vitamin E group (-36.4 +/- 17.7%) as compared to the *T. arjuna* group (-29.3 +/- 18.9%). It was concluded from this trial that, *Terminalia arjuna* tree bark powder has significant antioxidant action that is comparable to vitamin E. In addition, it also has a significant hypocholesterolaemic effect. (Antioxidant and hypocholesterolaemic effects of *Terminalia arjuna* tree-bark powder: a randomised placebo-controlled trial, J Assoc Physicians India 2001 Feb; 49:231-235)

Abeysekera et al. measured the antiradical and antilipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardioprotection *in vitro*, by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and deoxyribose damage protection assays, and (b) in vivo, by effects on lipid peroxidation. *Terminalia arjuna* aqueous freeze-dried extracts showed significant DPPH radical scavenging activity with EC (50) 8.3 +/- 0.3 microg/mL (similar to L-ascorbic acid). (Phytother Res. 2001 Sep; 15(6): 519-23.)

To explore the mechanism of action of arjunolic acid on cardiac protection in isoproterenol induced myocardial necrosis in rats, Puvanakrishnan R. et al, measured the anti-platelet activity, anticoagulant assays, electrocardiographic changes, serum marker enzymes, antioxidant status, lipid peroxide and myeloperoxidase (MPO) activity of arjunolic acid and compared the results with a potent cardioprotective drug, acetyl salicylic acid (ASA). Arjunolic acid treatment is also shown to prevent the decrease in the levels of superoxide dismutase, catalase, and glutathione peroxidase. (Mol Cell Biochem. 2001 Aug; 224 (1-2): 135-42.)

To explore the anti-bacterial potential of medicinal plants, Perumal SR et al., took a total of 34 plant species belonging to 18 different families, selected on the basis of folklore medicinal reports practiced by the tribal people of Western Ghats, India, and assayed for antibacterial activity against *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris*, and *Pseudomonas aerogenes* (gram-negative bacteria). 50 g of the powdered test material were extracted sequentially in 250 ml of hexane, diethyl ether, dichloromethane, ethyl acetate, methanol and water. These extracts at a concentration of 1000-5000 ppm were checked to determine the antibacterial potential by using the disc diffusion method. Of these 16 plants showed activity; among them *Cassia fistula*, *Terminalia arjuna* and *Vitex negundo* showed significant antibacterial activity against the tested bacteria. The bark of *T. arjuna* exhibited antibacterial activity only in dichloromethane, methanol and aqueous extracts against the bacteria tested at 1000–5000 ppm dosage; high activity was observed at 4000 and 5000 ppm against *P. aerogenes*. (Screening of 34 Indian medicinal plants for antibacterial properties Journal of Ethnopharmacology 62 (1998) 173–182)

Brief description of the figures and tables:

Fig. 1: Reducing potential of Ethyl acetate, Acetone, Ethanol, Methanol and water extracts from the bark of *Terminalia arjuna*.

Fig. 2 shows the DPPH free radical scavenging potential of Ethyl acetate, Acetone, Ethanol, Methanol and water extracts from the bark of *Terminalia arjuna*.

Fig. 3 shows antibacterial activity of *Terminalia arjuna* bark ethyl acetate, acetone, ethanol, methanol and water extracts (5mg/ml).

Fig. 4 shows antibacterial activity of *Terminalia arjuna* bark ethyl acetate

Fig. 5 shows growth of the bacterial strains on the LB, LB with 5 % DMSO and LB with 2 μg/ml ciprofloxacin as positive controls

Table 1 gives the readings of reducing potential of *Terminalia arjuna* bark ethyl acetate, acetone, ethanol, methanol and water successive extracts.

Table 2 gives the % inhibition values of DPPH free radical scavenging potential of *Terminalia arjuna* bark ethyl acetate, acetone, ethanol, methanol and water successive extracts.

Table 3 gives antibacterial activity of *Terminalia arjuna* bark ethyl acetate, acetone, ethanol, methanol and water extracts at 5mg/ml and 1 mg/ml concentrations.

Description

The medicinal properties of bark of *Terminalia arjuna* in alleviation of various health related disorders have been very well documented. In order to determine the cardio-protective principle(s), metabolic profiling of the *Terminalia arjuna* bark was done. Successive extraction was carried out of the bark of *Terminalia arjuna* with solvents in order from non-polar to polar starting with Hexane, Chloroform, Ethyl acetate, Acetone, Ethanol, Methanol and Water. The extracts obtained were subjected to various assays systems.

For evaluating anti-oxidation potential of the *Terminalia arjuna* bark extracts reducing potential and DPPH free radical scavenging activity were checked for and the levels of antioxidant was determined.

Plant material and extraction:

Powdered *Terminalia arjuna* bark was extracted by solvents sequentially in order from hexane, chloroform, ethyl acetate, acetone, ethanol, methanol and water. The individual fractions obtained were filtered and evaporated to dryness in vacuo.

Antioxidant assay:

The antioxidant activities of natural components may have reciprocal correlation with their reducing potentials. Several methods have been developed to measure the efficacy of dietary antioxidants as pure compounds or in food extracts. These methods focus on different mechanisms of the oxidant defense system i.e. scavenging active oxygen species and hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of lipid per-oxidation, or chelation of metal ions. In most of the cases irrespective of the stage in the non-enzymatic anti-oxidative activity (scavenging of free radicals, inhibition of lipid per-oxidation, etc.) is mediated by redox reactions.

1. Reducing power:

Various concentrations of the extracts (50 µl) were mixed with 200 µl of 0.2 M phosphate buffer, pH 6.5 and 200 µl of 1% potassium ferricyanide, and then incubated at 50 °C for 20 min 10% trichloroactetic acid (250 µl) was added to the mixture and centrifuged at 3000x g for 10 min at room temperature. Of the resulting supernatant, 500 µl was taken and mixed with 500 µl of 0.1% ferric chloride then incubated at 37 °C for 10 min. The absorbance at 700 nm was measured. This assay was done in triplicate. Increased absorbance indicated increased reducing power.

2. DPPH Scavenging Effect

This method is based on the reduction of DPPH, a stable free radical. Due to the odd electron of DPPH, it gives a strong absorption maximum at 517 nm by visible spectroscopy (purple color). As the odd electron of the radical becomes paired off in the presence of hydrogen donor, that is, a free-radical scavenging antioxidant, the absorption strength is decreased, and the resulting de-coloration is stoichiometric with respect to the number of electrons captured. This reaction has widely been used to evaluate the anti-oxidative activity of food and plant extracts.

Reactions were performed in 1.25 ml of methanol containing 0.5 mM freshly made DPPH and various amounts of the extract. Reaction mixtures were incubated at 37 °C for 30 min, and the absorbance at 517 nm was measured. This assay was done in triplicate.

Antibacterial assay:

For evaluating antibacterial potential of the *Terminalia arjuna,* successive Ethyl acetate, Acetone, Ethanol, Methanol and Water extracts from bark of *Terminalia arjuna* were tested against 11 bacterial strains .

Cultures tested:

Testing of anti-microbial potential was done against eleven bacterial strains (Gram negative: *Escherichia coli* ATCC-10536, *Pseudomonas aeruginosa* ATCC-9027, *Klebsiella pneumoniea* ATCC-10031, *Bordetella bronchiseptica*

ATCC-4617; Gram Positive: *Staphylococcus aureus* ATCC-29737, *Streptococcus fecalis* ATCC-8043, *Micrococcus luteus* ATCC-9341, *Bacillus subtilis* ATCC-6633, *Bacillus cereus* ATCC-11778, *Bacillus pumilus* ATCC-14884, *Staphylococcus epidermidis* ATCC-6358) were selected from the microorganisms given in United states Pharmacopoeia (2000), British Pharmacopoeia (1993) and Indian Pharmacopoeia (1996) for anti-microbial assays.

Agar streak method:

A stock of 100 mg/ml of Ethyl acetate, Acetone, Ethanol, Methanol and Water successive extract from the bark of *Terminalia arjuna* was prepared in DMSO. To determine the antibacterial potential extracts at a concentration of 5 mg/ml and 1 mg/ml were added to 30 ml of LB agar medium. After the medium was solidified, overnight grown 11 bacterial strains mentioned were taken in loop and streaked on the medium. The plates were incubated at 37 °C for 24 hrs after which the bacterial growth was monitored. Suitable controls were maintained with the extracts and the microorganisms. Ciprofloxacin (2 µg/ml) served as positive control.

Results and Discussions:

Antioxidation Potential:

The reducing potential of the bark extracts of *Terminalia arjuna* has been shown in Figure 1. It is seen that with the increase in the extract concentration there is increase in the reducing power. The concentration required to attain one absorbance unit at 700 nm were 0.081 mg/mL for ascorbic acid, 0.287 mg/mL for acetone and ethanol, 0.4 mg/mL for methanol and 0.431 mg/mL for ethyl acetate. At higher concentration of i.e. at concentration of 1.25 mg/mL the reducing power of both acetone and ethanol extract was higher then ascorbic acid. The presence of high reducing power indicates the presence of compound that could donate electrons and could react with free radicals to convert them to more stable products and to terminate radical chain reaction.

The radical scavenging activity of the *Terminalia arjuna* bark extracts was determined against the DPPH radical in spectrophotometric assay. The DPPH radical was quenched by the antioxidants as indicated by an acceleration of the decay of the absorbance signal (515 nm). It was found that the reduction of DPPH radical was dose dependent. It was seen that IC50 of acetone extract was 25 µg/mL less than that of ascorbic acid 26.4 µg/mL. In case of the other extracts the IC50 of ethyl acetate, ethanol, methanol and water was determined as 52.8 µg/mL, 36.8 µg/mL, 34.3 µg/mL and 46.4 µg/mL respectively.

Antibacterial Potential:

Table 3 enumerates the effect of the antibacterial properties of different solvent and aqueous extracts of bark extract of *Terminalia arjuna* against the 11 bacterial stains tested.

It is observed that at concentration of 5 mg/ml ethyl acetate extract exhibited a broad antibacterial inhibiting growth of 9 of the 11 bacterial strains tested (Fig 1 and 3). It was found very effective against the gram-positive bacteria showing inhibition of all the seven gram positive strains tested. Whereas acetone, ethanol, methanol and water extract showed inhibition against *B. bronchiseptica, S. aureus, S. fecalis* and *M. luteus*.

At concentration of 1 mg/ml ethyl acetate showed antibacterial activity against *B. bronchiseptica, S. aureus* and *S. fecalis*. Acetone extract showed complete inhibition of growth of *S. aureus* and *S. fecalis* whereas showed partial growth inhibition against *B. bronchiseptica*. Ethanol, methanol and water extract showed inhibition against only *S. aureus*.

Claims

1. A method of arriving at a formulation from *Terminalia arjuna,* effective in the treatment of health related disorders like Cardiovascular disorders, *Diabetes mellitus*, Obesity.

2. A method of arriving at a formulation from *Terminalia arjuna,* effective as anti-microbial agent.

3. A claim as in claim 1, wherein the metabolites isolated and characterized from the formulation can be used individually in the treatment of health related disorders.

4. A claim as in claim 1, wherein the metabolites isolated and characterized from the formulation can be used individually in the treatment of cardiovascular disorders.

5. A claim as in claim 2, wherein the metabolites isolated and characterized from the formulation can be used individually as antimicrobial agents.

6. A claim as in claim 1 & 4 wherein the series of metabolites isolated form *Terminalia arjuna* exhibited significant antioxidant potential, as indicated by the DPPH free radical scavenging potential.

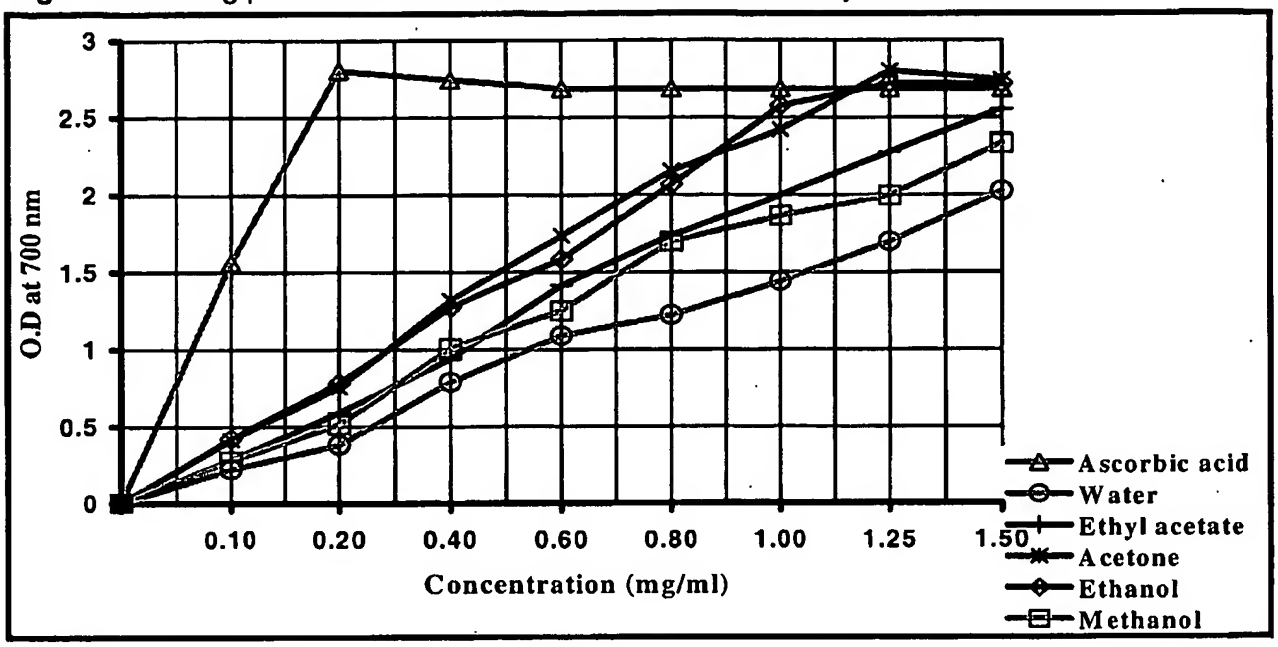
7. A claim as in claim 6, wherein metabolite(s) are effective in the treatment of age-related diseases like amyloidosis, acute pancreatitis, arthritis, cancer, inflammatory bowel disease, senile dementia, retinal degeneration and senile cataract.

8. A claim as in Claims 3, wherein, the series of metabolites can form a major source of new therapeutics and nutraceuticals

9. A claim as in claim 1 & 3, wherein the formulation can be administered orally, parenterally, intravenously, and intradermal administration.

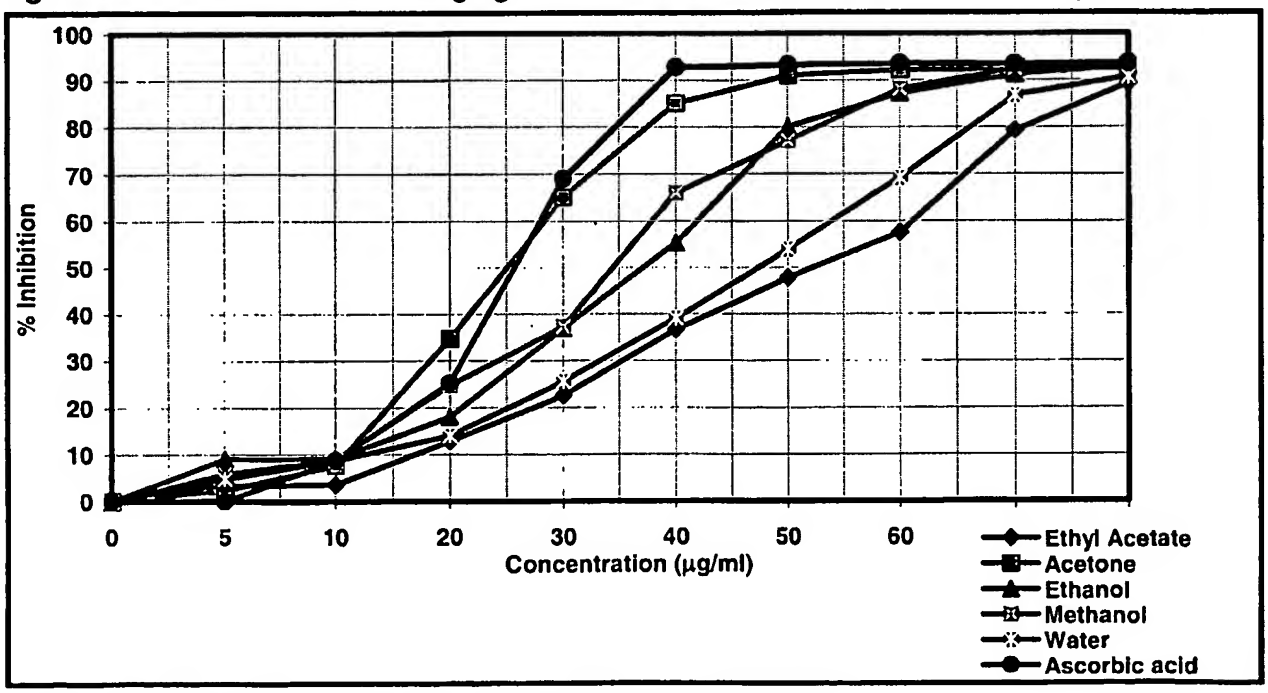
10. A claim as in claim 9, wherein the dosage can be administered in the form of tablet, capsule, powder, pill, solution, syrup, suspension, emulsion, and granules.

Fig. 1: Reducing potential of bark extracts from *Terminalia arjuna*. bark extracts.



Each value is expressed as mean±standard deviation (n=3).

Fig. 2: DPPH free radical scavenging effect of bark extracts from *Terminalia arjuna*.



Each value is expressed as mean±standard deviation (n=3).

Fig 3. Antibacterial activity of *Terminalia arjuna* bark ethyl acetate, acetone, ethanol, methanol and water extracts (5mg/ml).

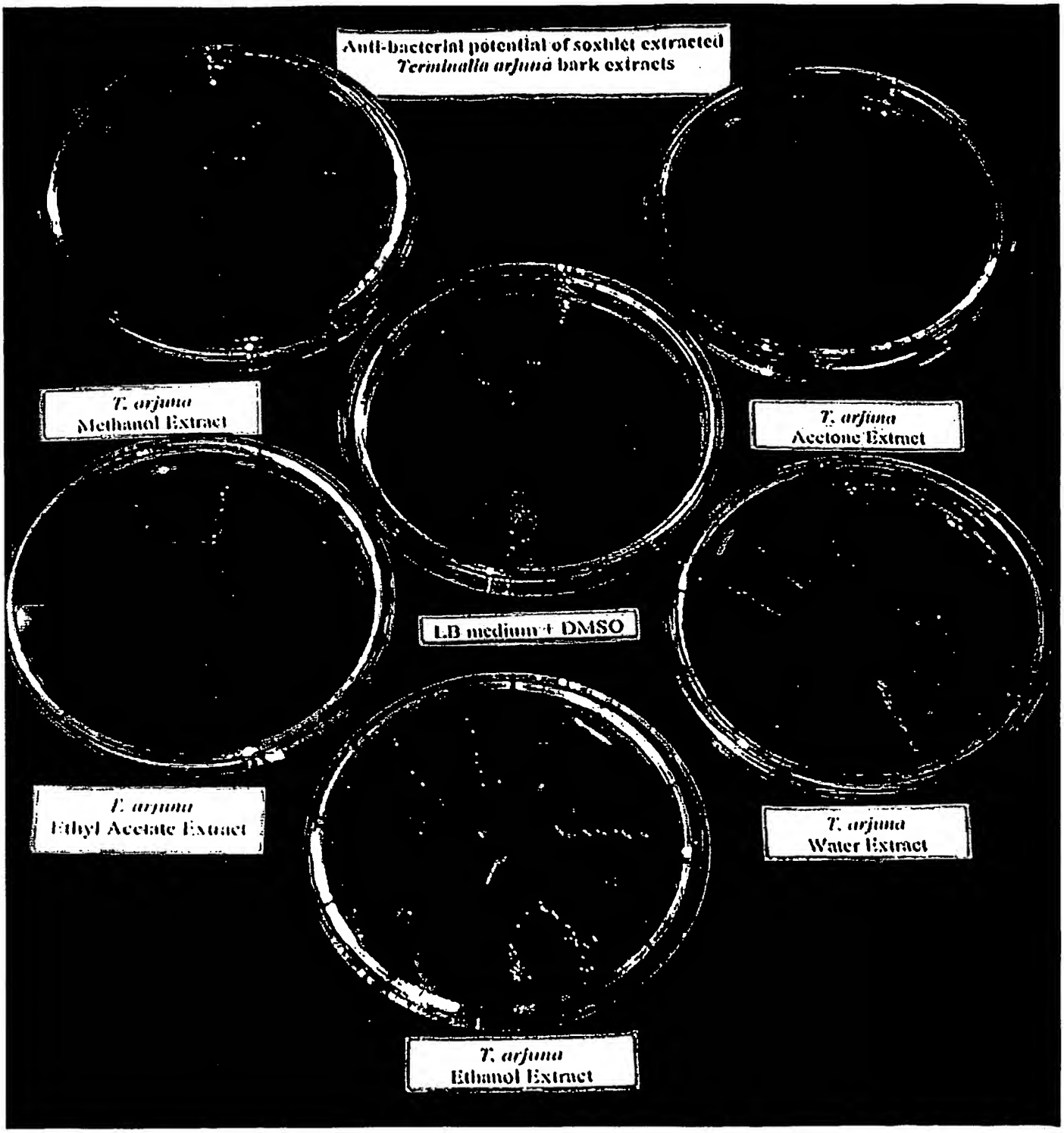


Fig 4: Antibacterial activity of *Terminalia arjuna* bark ethyl acetate

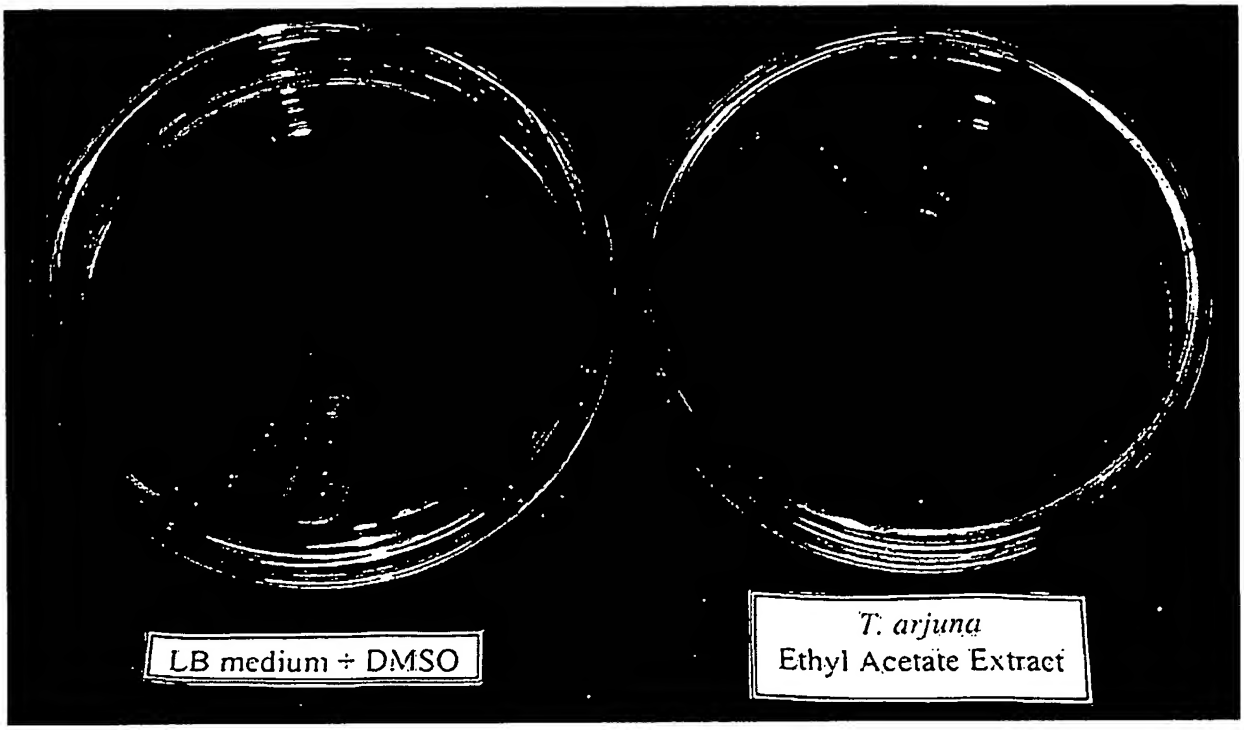


Fig 5: growth of the bacterial strains on the LB, LB with 5 % DMSO and LB with 2 µg/ml ciprofloxacin as positive controls

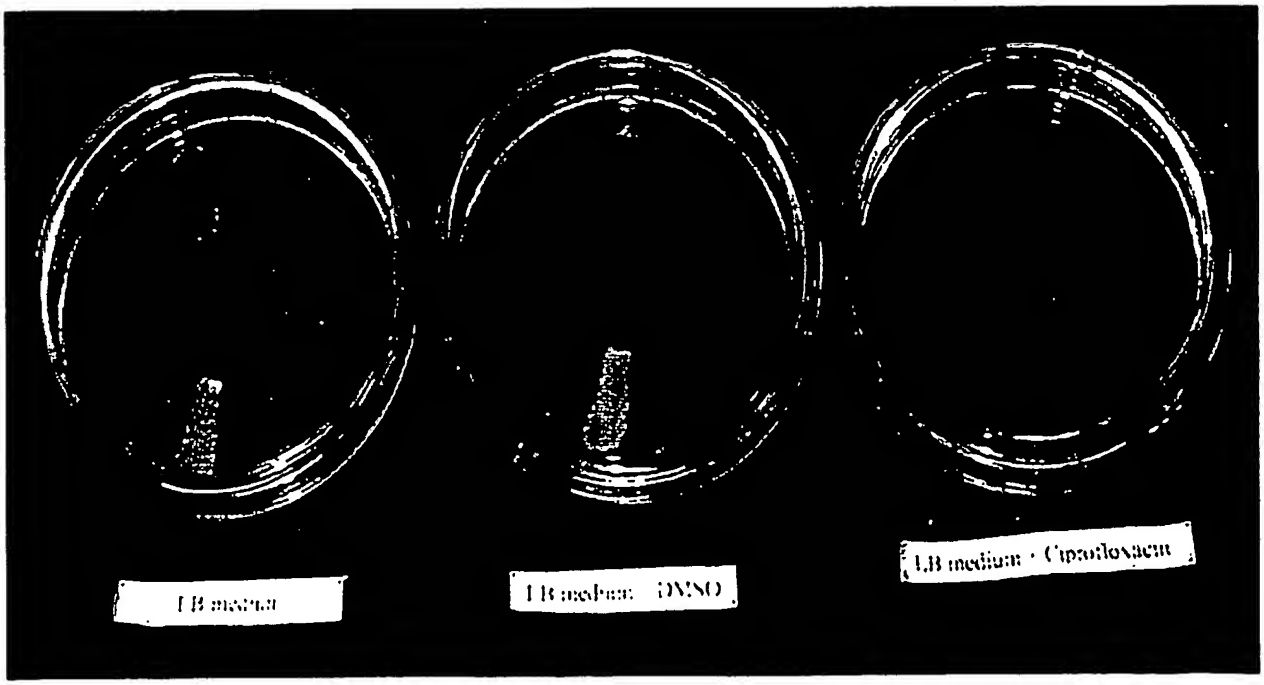


Table 1: Reducing potential of *Terminalia arjuna* extracts.

Sr. No	Conc. (m g/ml)	O.D at 700 nm					
		Ethyl acetate	Acetone	Ethanol	Methanol	Ascorbic acid	Water
1.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.	0.10	0.281± .017	0.414±.014	0.421±.047	0.276±0.018	1.562±.076	0.221± 0.007
3.	0.20	0.589±.023	0.754±.016	0.781±.076	0.513±.020	2.803±.088	0.380± 0.055
4.	0.40	0.941±.020	1.318±.055	1.273±.082	1.008±.051	2.774±.128	0.789± 0.026
5.	0.60	1.415±.048	1.737±.200	1.584±.177	1.252±.181	2.686±.042	1.089± 0.046
6.	0.80	1.737±.256	2.154±.070	2.066±.143	1.698±.027	2.686±.042	1.222± 0.072
7.	1.00	1.998±.073	2.420±.125	2.575±.083	1.884±.109	2.686±.042	1.439± 0.120
8.	1.25	2.277±.084	2.802±.064	2.717±.111	1.995±.122	2.686±.042	1.697± 0.121
9.	1.5	2.554±.072	2.774±.140	2.717±.121	2.334±.056	2.686±.042	2.023± 0.089

Each value is expressed as mean±standard deviation (n=3).

Table 2: DPPH free radical scavenging effect of bark extracts from *Terminalia arjuna*

Sr. No	Conc. (µg/ml)	% Inhibition					
		Ethyl acetate	Acetone	Ethanol	Methanol	Water	Ascorbic acid
1.	0	0	0	0	0	0	0
2.	5	3.4	2.3	9	5.8	4.8	0
3.	10	3.6	7.7	9.1	8.9	8.8	8.8
4.	20	12.8	34.6	18	24.8	14	25.2
5.	30	22.5	65	37	37.3	25.6	68.9
6.	40	36.5	85.1	55.3	66	39.1	92.8
7.	50	47.8	91.1	80.1	77	53.9	93.3
8.	60	57.5	92.3	87.2	88.1	69.2	93.6
9.	80	79.3	92.5	91.2	92.5	86.8	93.5
10.	100	89.3	92.8	93	92.5	90.8	93.7

Each value is expressed as mean±standard deviation (n=3).

Table 3. Antimicrobial activity of soxlet extracted *Terminalia arjuna* bark extracts:

Sr. No.	Organism	Extracts										Control		
		Ethyl Acetate		Acetone		Ethanol		Methanol		Water		LB	LB+ DMSO (5%)	LB + Cipro-floxacin (2 µg/ml)
		1mg/ml	5 mg/ml	1mg/ml	5 mg/ml	1mg/ml	5mg/ml	1mg/ml	5 mg/ml	1mg/ml	5 mg/ml			
	Gram Negative													
1.	*E. coli*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
2.	*P. aeruginosa*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
3.	*K. pneumoniea*	+++	-	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	-
4.	*B. bronchiseptica*	-	-	+	-	+++	-	+++	-	+++	-	+++	+++	-
	Gram Positive													
5.	*S. aureus*	-	-	-	-		-		-		-	+++	+++	-
6.	*S. fecalis*	-	-	-	-	+++	-	+++	-	+++	-	+++	+++	-
7.	*M. luteus*	+++	-	+++	-	+++	-	+++	-	+++	-	+++	+++	-
8.	*B. subtilis*	+++	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
9.	*B. cereus*	+++	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
10.	*B. pumilus*	+++	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
11.	*S. epidermidis*	+++	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-

+++; abundant growth, +; growth; -, no growth

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB03/03678

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 35/78; C01B 21/26, 31/02, 9/08
US CL : 424/769, 404, 451, 464, 489; 514/824, 825, 866, 878, 879, 909, 912

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/769, 404, 451, 464, 489; 514/824, 825, 866, 878, 879, 909, 912

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MUNASINGHE et al. Antiradical and Antilipoperoxidative Effects of Some Plant Extracts used by Sri Lankan Traditional Medical Practitioners for Cardioprotection. Phytotherapy Research. September 2001, Vol 15. No. 6, pages 519-523, entire document.	1, 3, 4 and 6-10
X	MILLER, A.L. Botanical Influences on Cardiovascular Disease. Alternative Medicine Review. December 1998, Vol 3. No. 6, pages 421-431, especially pages 425-428.	1-10
X	SAMY et al. Screening of 34 Indian Medicinal Plants for Antibacterial Properties. Journal of Ethnopharmacology. September 1998, Vol 62. No. 2, pages 173-178, especially pages 175, 177 and 180.	1-3, 5 and 8-10.
X	KANDIL F.E. et al. A Tannin Anti-cancer Promotor from Terminalia arjuna. April 1998, Vol 47. No. 8, pages 1567-1568, entire document.	1, 3 and 8-10
X	DWIVEDI, S. et al. Beneficial Effects of Terminalia arjuna in Coronary Artery Disease. Indian Heart Journal. September-October 1997, Vol. 49. No. 5, pages 507-510, entire document.	1, 3, 4 and 8-10.
X	RAM et al. Hypocholesterolaemic Effects of Terminalia arguna Tree Bark. Journal of Ethnopharmacology. Vol 55. No. 3, pages 165-169, entire document.	1, 3 and 8-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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26 February 2004 (26.02.2004)	09 NOV 2004
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